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In re Application of

Atty. Docket No.: 26068-08D

Anderson

Serial No.: 09/891,064

Art Unit: 1644

Filed: June 25, 2001

Examiner: P. Nolan

Title: Human Occludin, Its Uses and Enhancement of Drug Absorption Using Occludin Inhibitors

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION OF DR. JAMES M. ANDERSON**

I, JAMES M. ANDERSON, hereby declare as follows:

1. I am currently Professor and Chair in the Department of Cell and Molecular Physiology, School of Medicine, University of North Carolina at Chapel Hill. I received my M.D. from Harvard Medical School (1983) and my Ph.D. from Harvard University (1979). Previously, I had been involved in research and teaching in Medicine, Physiology and Cell Biology at Yale University from 1988 to 2002 (14 years). I am a member of the American Society for Cell Biology, American Physiological Society, American Society for Clinical Investigation, American Society for Advancement of Science and a number of other societies set forth in my Curriculum Vitae (C.V.), a copy of which is appended hereto as Exhibit A. I have

authored or co-authored over 62 peer-reviewed publications in addition to invited review articles, book chapters and books. My service on various National Committees, committees at Yale University and Yale Medical School and UNC of Chapel Hill School of Medicine are summarized in the attached C.V.

2. I am actively involved in the research disclosed in, and am named as a co-inventor of the above-identified application and its parent applications, and am therefore well aware of their contents.
3. I have reviewed the above-referenced application and the office action mailed January 23, 2003. I submit this declaration in connection with the office action. More specifically, I submit this declaration in connection with the claim rejections based on "Interspecies Diversity of the Occludin Sequence: cDNA Cloning of Human, Mouse, Dog, and Rat-Kangaroo Homologues," Ando-Akatsuka et al., The Journal of Cell Biology, Vol. 133, No. 1, April 1996, pp. 43-47 (hereinafter referred to as the Ando-Akatsuka publication).
4. The publication date of the Ando-Akatsuka publication was April, 1996. The sequence of human occludin reported in the Ando-Akatsuka publication paper was also available on the Internet through the National Center for Biotechnology Information (NCBI) on February 1, 1996 under accession number U49184.

5. My co-inventors and I isolated and sequenced the cDNA for human occludin and deduced its amino acid sequence at least as early as 1995, which is before the publication of the Ando-Akatsuka publication. Thus, my co-inventors and I had possession of the currently pending claims before the earliest publication date of the Ando-Akatsuka publication.
6. The attached documentation establishes that we isolated and sequenced the cDNA sequence for human occludin at least as early as 1995. This is shown on pages 23, 40, and 78 of Dr. Christina Van Itallie's laboratory notebook, copies of which are attached hereto as Exhibit B. The notebook pages are dated prior to the earliest publication date of the Ando-Akatsuka publication, but the dates have been redacted to maintain the secrecy of the date of my invention.
7. Page 23 of Dr. Christina Van Itallie's laboratory notebook is entitled "Plasmid preps on 1, 5, 7 for sequencing, Northerns, etc." Plasmids 1, 5, and 7 contained cDNA sequences of human occludin obtained by screening a human cDNA library. Lines 7 and 8 from the bottom refer to DNA sequencing reactions of clones 1 and 7, which were submitted to the Yale Sequencing Facility for automated sequencing.
8. Ja1OCT7 designates: James Anderson clone 1 of human occludin sequenced by priming the plasmid with the T7 primer (hereinafter "clone 1"). Ja7OCT7 designates: James Anderson clone 7 of human occludin as sequenced by priming the plasmid with the T7 primer (hereinafter "clone 7").

9. Clone 1 encodes the correct full-length human occludin. Clone 7 lacks sequence encoding the N'-terminal 32 amino acid residues. We submitted the sequence of clone 7 in Figure 2 of our U.S. Provisional Application. We recognized that clone 1 contained the correct N'-terminal sequence after release of NCBI-accession number U49184, as acknowledged at the top of page 78 in Dr. Van Itallie's notebook. Clone 1 overlaps clone 7 from amino acid residues 33 to 522 of SEQ. ID. NO. 2. Both clones 1 and 7 code for the extra-cellular loops of interest in the present application. The extra-cellular loops are residues 89 to 138 and residues 196 to 246 of SEQ. ID. NO. 2.

10. We were working with both clones 1 and 7 prior to the earliest publication date of the Ando-Akatsuka publication. By the filing date of our U.S. Provisional Application, we were using clone 7 in our continuing research.

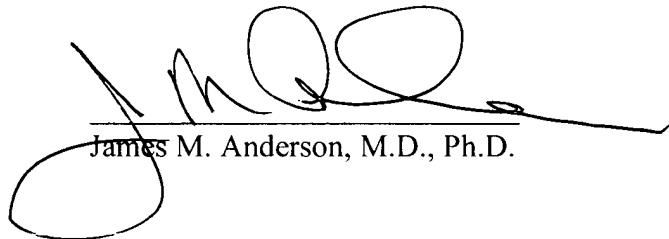
11. Page 40 from Dr. Van Itallie's notebook is also dated prior to the earliest publication date of the Ando-Akatsuka reference. This page is entitled "Make full length ocl clone for expression". As noted, clones 1 and 7 differ at the 5' end of their coding regions. Clone 1 encodes the correct full length human occludin. At the time we simultaneously pursued the possibility that clone 7 might contain the correct 5' end. In the protocol described on page 40, Dr. Van Itallie is ligating the 5' end of clone 7 onto the 3' end of clone 1 at a shared Bgl II site and cloning them into a mammalian expression vector. Page 40 also shows this protocol continued on a later date.

12. We completely identified the sequence of human occludin as presently claimed prior to the earliest publication date of the Ando-Akatsuka publication. Thus, my co-inventors and I invented the subject matter of the present application, as presently claimed, before it was described in the Ando-Akatsuka publication.

13. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Chapel Hill, NC, United States

Date: 6-16-03



A handwritten signature in black ink, appearing to read "James M. Anderson, M.D., Ph.D.", is written over a horizontal line. The signature is fluid and cursive, with a large, stylized 'J' at the beginning.

**Jam s M. Anderson, Ph.D., M.D.****P rsonal**

Born: July 17, 1952 - Champaign, Ill, USA  
 Work Address: The University of North Carolina at Chapel Hill  
                   6312 Human Biomolecular Research Building  
                   Campus Box 7545  
                   Chapel Hill, North Carolina 27599-7545  
 Work Phone: (919)966-6411  
 Fax: (919)966-6413  
 E-mail Address: jandersn@med.unc.edu

**Present Position**

Professor and Chair  
 Department of Cell and Molecular Physiology  
 The University of North Carolina at Chapel Hill

**Education**

B.S.	Biology	Yale College, New Haven, CT	1974
Ph.D.	Biology	Harvard University	1979
M.D.		Harvard Medical School	1983
M.S.	Science	Yale University (honorary)	1998

**Clinical Experience**

Intern/Resident, Yale-New Haven Hospital, New Haven, CT	1983-86
Postdoctoral Fellowship, Hepatology, Yale School of Medicine, New Haven, CT	1986-89
Diplomat, American Board of Internal Medicine	1986 -
Connecticut State Medical License - 027065	1985 -
Attending Physician, Yale-New Haven Hospital Internal Medicine and Hepatology	1988 - 02
Attending Physician, West Haven Veteran's Administration Hospital Internal Medicine and Hepatology	1988 - 02

**Prof ssional Experience**

Yale School of Medicine	
Assistant Professor of Internal Medicine	1988 - 91
Associate Professor of Internal Medicine and Cell Biology	1991 - 98
Associate Professor (without term)	1996 - 98
Chief, Section of Digestive Diseases	1996 - 02
Professor of Medicine and Cell Biology	1998 - 02
The University of North Carolina at Chapel Hill	
School of Medicine	
Professor and Chair, Cell and Molecular Physiology	2002 -

**Honors and Recognition**

Individual NRSA	1986 - 88
Terry Kirgo Memorial Fellowship, American Liver Foundation	1987 - 88
Lucille P. Markey Scholar Award in Biomedical Science	1988 - 94
The Dean's Young Faculty Award, Yale School of Medicine	1991

James M. Anderson, Ph.D., M.D.

American Society for Clinical Investigation, elected member  
Interurban Clinical Club (Boston/NY/New Haven/Phili/Baltimore), elected member  
American Association of Physicians, elected member

1994 -  
1994 - 02  
1999 -

### Professional Affiliations

American Society for the Advancement of Science  
American Association for the Study of Liver Diseases  
American Association of Physicians  
American Gastroenterological Association & Gastroenterology Research Group  
American Physiological Society  
American Society for Cell Biology  
American Society for Clinical Investigation  
Association of Subspecialty Professors  
International Association for the Study of Liver Diseases

### Editorial Boards

Gastroenterology 1999 - 04  
Journal Clinical Gastroenterology 1999 - 04  
Ad Hoc Referee

### Committees and Activities

#### National Committees

Advisory Board Member 1996 -  
Harvard Digestive Diseases Research Core Center - (NIH)  
University of Pennsylvania School of Medicine, 1998 - 03  
Center for Studies of Digestive and Liver Diseases - (NIH) 1992 - 96  
Research Committee, Am. Gastro. Assoc. 1996 - 99  
Research Committee, Am. Assoc. Study Liver Diseases 1996 - 97  
Selection Committee, Life Sciences Research Foundation (Princeton, NJ) 1996 - 97  
NIH NIGMS Biomedical Research & Research Training Committee 1996 - 00  
BRT-A Study Section 2001  
FASEB Research Conference, GI Track VIII, Co-organizer, 2001  
Experimental Biology 2001, Symposium Organizer 2001  
Ph.D., DVM, MD/PhD Committee, Am Gastro Assoc 2001 - 03  
Chair 2003 - 05  
Organizer, Special Interest Subgroup Meeting, 14 Dec. 2002 2002  
Annual Meeting of the Am Soc for Cell Biology, San Francisco, CA 2003-  
Association of Chairs of Departments of Physiology 2004-07  
Membership & Diversity Council, Am Gastro Assoc

#### Yale University

Biological Sciences Advisory Committee 1998 - 00  
Tenure and Appointments Committee for the Biological Sciences 1998 - 00

#### Yale Medical School

Co-Director, L.P. Markey Physician-Scientist Training Program 1991 - 96  
M.D./Ph.D. [MSTP] Selection Committee 1990 - 02  
Boyer Center Junior Faculty Program Selection Committee 1994 - 95  
Anna Fuller Molecular Oncology Fellowship Selection Committee 1994 - 98  
Advisory Board, Yale Critical Technologies Program 1995 - 97  
Member, Yale Comprehensive Cancer Center 1995 - 02  
Advisory Committee, Center for Cell Imaging - Cell Biology 1996 - 97

James M. Anderson, Ph.D., M.D.

Internal Selection Committee, HHMI Investigator Nominees	1996 & 01
Search Committee, Chair of Cellular and Molecular Physiology Department	1998 - 99
Liver Transplantation Steering Committee	1996 - 02
New Research Building Space Allocation Committee	1998 - 02

**Yale Department of Internal Medicine**

Director, Research Pathway	1993 - 02
Residency Selection Committee	1990 - 02
Space Allocation Committee	1999 - 02
Search Committee, Chief of Medical Oncology	2000 - 01

**Yale Division of Digestive Diseases**

Chief	1996 - 02
Assoc. Director, Yale Liver Center - (NIH)	1998 - 02
Executive Committee, Yale Liver Center - (NIH)	1993 - 02
Director, Investigative Hepatology Training Grant - (T32, NIH)	1999 - 02

**UNC at Chapel Hill School of Medicine**

Basic Science Chairs Committee	2002-
Advisory Committee for the School of Medicine	2002-
Lineberger Comprehensive Cancer Center, Member	2002-
Gottschalk Award Nominating Committee	2002-
Scientific Misconduct Case Inquiry Team	2002
Medical-Scientist Training Program, Executive Committee	2002-
Cell & Molecular Biology Training Program (NIH-T32), Executive Committee,	2002-
Associate Director, Center for Gastrointestinal Biology and Disease (NIH-P30)	2003-
Interdisciplinary Biomedical Sciences Graduate Program, Committee Member	2002-
Faculty Salary Equity Committee	2003-

**UNC Department of Cell & Molecular Biology**

Graduate Committee (Cell & Molecular Physiology), co-Chair	2002-
Faculty Recruitment Committee, Chair	2002-
Director, Weekly Seminar Series	2002-
Research Day, Director	2002-

**Postdoctoral Trainees**

**Yale School of Medicine**

Elizabeth Willott, Ph.D.	1988 - 90	<u>Present Position</u>
Michael Fallon, M.D.	1989 - 93	Research Faculty, Univ. Arizona
Maria Susana Balda, Ph.D.	1990 - 94	Prof. of Medicine, Univ. Alabama - Birmingham
Barry Slitzky, M.D.	1990 - 92	Research Faculty, Univ. London
David Rimm, M.D., Ph.D.	1990 - 91	Assoc. Prof. of Pathology, Yale
Stuart Levin, M.D.	1992 - 94	
Alan S. Fanning, Ph.D.	1993 - 96	Research Faculty, Yale University
Lynne Lapierre, Ph.D.	1994 - 97	Research Faculty, Cell Biology, Vanderbilt
Zenta Walther, M.D., Ph.D.	1997 - 02	Asst. Prof. of Pathology, YSM
Christoph Rahner, M.D.	1998 - 01	Asst. Prof. Surgery, Yale University
Rolando Medina, Ph.D.	1999 - 00	Biotech Patent Lawyer
Laura Mitic, Ph.D.	2000 - 02	Postdoctoral Associate, UCSF

**Graduate Students**

**Yale University**

Alexander Brecher, BS MSTP/Cell Biology	1994 - 99	Dermatology Resident, New York University
Laura Mitic, BS	1996 - 00	Postdoctoral Associate, UCSF

James M. Anderson, Ph.D., M.D.

Cell Biology

Danette Daniels, BS

1995 - 99

Postdoctoral Associate, Stanford University

Co-advisor (Alex Brunger)

Molecular Biology & Biophysics

Oscar Colegio, BS

2000 -

MSTP/Cell Biology

**Invited Research Speaker (selected)**

Invited Plenary Speaker, Int. Union of Physiol. Sci., Glasgow, Scotland, 3 Aug. 1993

L.P. Markey Trust Symposium, San Diego, CA, Sept. 1993

SU New York Stony Brook MSTP Program, 11 May 1994

Medical College of GA, Inst. of Molecular Medicine, May 1994

Developmental Biology Center, UC Irvine, 13 June 1994

R.W. Johnson Medical School, Cell and Dev. Biol., New Jersey, Feb. 1995

University Speaker, Leicester, England, 4 April 1995

Germany GI Society, State of the Art, Berlin, 16 Sept. 1995

Iberoamerican Soc. Cell Biol., Mexico City, 7 Oct. 1995

Keystone Symposium, Intercellular Junctions, March 1996

Boehringer Ingelheim Fonds International Conference, Titisee, Germany

State-of-the-Art, "Cell Junctions and Disease," Oct. 1996

University of Colorado, Denver, Physiology Dept., Nov. 21, 1996

Harvard Medical School, MGH Gastroenterology Section, Boston, MA, Feb. 25, 1997

Invited Speaker, Falk Symposium, Freiburg, Germany, 1 Oct. 1997

Invited Plenary Speaker, American Society Nephrology, San Antonio, TX, 4 Nov. 1997

Symposium Speaker, MGH/Harvard, Mucosal Immunology, Boston, MA, 11 Nov. 1997

Invited Speaker, Center for the Study of Basic Mechanisms of Inflammatory Bowel Disease,  
MGH/Harvard, Nov. 14-15, 1997

Biochemistry Department, UT San Antonio, 6 March 1998

3rd Intl. Malpighi Symposium, Monterey, CA, April 1998

Invited Plenary Speaker, Annual FASEB Meeting, Washington, DC, April 1998

Symposium Speaker, AGA/Digestive Disease Week, New Orleans, LA, 19 May 1998

Medical Grand Rounds, Hospital of St. Raphael's, New Haven, CT, 2 June 1998

Invited Speaker, Falk Symposium, Titisee, Germany, 17 Oct. 1998

GI Grand Rounds, MGH/Harvard Medical School, 2 Feb. 1999

FASEB, GI Tract, Copper Mt., 25-30 July 1999

Physiology Dept. University of Texas Southwestern, Sept. 27, 1999

ASCB MAGUK Symposium, Washington, DC, 11 Dec. 1999

Keystone Symposium Chair, Mucosal Immunity, Taos, NM, 18-22 Jan. 2000

Keystone Symposium, Intercellular Junctions, Feb. 2000

Soc. Pediatric Pathology, New Orleans, LA, 25 Mar. 2000

Yale Cell Biology Department Retreat, 7 April 2000

Research Lecture, Jichi Medical School, Utsunomiya, Japan, 7 Sept. 2000

4th US-Japan GI Meeting Program, Tokyo, Japan, 8 Sept. 2000

Asahikawa GI and Hepatology Symposium, Asahikawa Medical College, Otaru, Japan, 10 Sept. 2000

GI Symposium, Kyoto Medical School, Kyoto, Japan, 12 Sept. 2000

10th Annual Arias Symposium, American Liver Foundation, Boston, MA, 25 Oct. 2000

Medicine Department, Mt. Sinai School of Medicine, 16 Jan. 2001

Experimental Biology 2001 - Symposium Chair, Tight Junction: Convergence of Molecular and Physiologic  
Insights, Orlando, FL, 1 April 2001

Gordon Research Conference - Cell Contact, Andover, NH, June 2001

AstraZeneca - Mucosal Defense Mechanisms, Gothenburg, Sweden, June 2001

FASEB Research Conference, GI Track VIII (co-organizer), August 2001

Cell & Molecular Physiology Dept., UNC-Chapel Hill, 25 Sept. 2001

Yale Pathology Department Grand Rounds, 18 Oct. 2001

Canadian Gastroenterology Society, Montreal, 3 Feb. 2002

James M. Anderson, Ph.D., M.D.

MD-PhD Retreat, UNC at Chapel Hill, Wilmington NC, 3 Aug. 2002  
European Intestinal Transport Group, Egmond aan Zee, NL, 28 Sept. 2002  
Dept. of Physiology, Northwestern School of Medicine, Chicago, IL, 10 Oct. 2002  
Dept. of Cell Biology, UNC, Chapel Hill, 23 Oct. 2002.  
Am. Soc. for Nephrology, Ann. Meeting, symposium speaker, 3 Nov. 2002  
Dept. of Pharmacology, UNC-Chapel Hill, Chapel Hill, NC, 3 Dec. 2002  
USC School of Medicine, Pulmonary Division, 13 Dec. 2002  
Co-organizer, ASCB meeting on Tight Junction, San Francisco, CA 14 Dec. 2002  
NIH-NIEHS, Chapel Hill, NC, 8 Jan. 2003  
Transatlantic Airway Conference, Key Biscayne, FL, 15 Jan. 2003  
Annual Higuchi Research Seminar, Univ. Kansas Pharmaceutical Chemistry, 4 May 2003

### **Scientific Advisory Boards**

Scientific Advisory Board, WEST Pharmaceutical Services, Lionville, PA, 1994 - present  
GI Transport Advisory Board, ALZA Corporation (J&J), Mountainview, CA, 2000  
Scientific Advisory Board, Nastech, Seattle, WA, 2003-present

### **Extramural Grants**

#### Ongoing Research Support

RO1 DK 45134 Anderson (PI) NIH/NIDDK <i>Molecular Analysis of Tight Junctions in Liver and Gut.</i> The goal of this grant is to understand the molecular basis for control of paracellular transport in normal and diseased epithelia with the long-term goal of manipulating these properties for therapeutic purposes. Role: PI	04/01/03 – 03/31/08
PO1 DK055389 Morrow (PI) NIH/NIDDK <i>Cell and Molecular Pathobiology of Renal Disease.</i> The overall goals of this project are to understand epithelial cell organization including membrane trafficking, myosin motor and angiogenesis in the kidney and in response to injury. Subproject 4 focuses on the response of tight junctions to reversible ischemia. Role: PI on Subproject 4	12/01/98 - 11/30/03
RO1 DK Anderson (PI) NIH/NIDDK <i>ZO-1 and cytoplasmic scaffolding at the tight junction.</i>	04/01/03 – 03/31/08

#### Completed Funding (last 3 years)

P30 DK34989 Boyer (PI) NIH/NIDDK <i>Digestive Diseases Research Core - Yale Liver Center</i> Role: Associate Director of Center and Director of the Molecular Biology Core	07/01/99 - 06/30/04
T32 DK07356 Anderson (PI) NIH/NIDDK <i>Investigative Training in Hepatology</i> Role: Director	07/01/99 - 06/30/04
PO1 CA66263 Bryant (PI, UC Irvine)	07/01/95 - 06/30/00

James M. Anderson, Ph.D., M.D.

NCI

*Membrane Associated Guanylate Kinase Homologs*

The goals of this grant are to study a class of proteins called MAGUKs, which are important in organizing membrane domains. A range of methods are used including genetics (*Drosophila*, *C. elegans* and mice), Cell Biology and x-ray crystallography to define the protein structure, interactions and function. Project 4 is focused on the mammalian MAGUKs CASK, hDlg and ZO-1. Much of the work focused on the biology of PDZ domains and work on ZO-1 is focused on its intramolecular domain interactions and how these regulate binding to other proteins.

Role: PI on Subproject 4

## Bibliography

### Original Peer-Reviewed Articles:

1. Anderson, J.M., Kleinhaus, A., Manuelides, L. and J.W. Prichard. 1974. Beveled dual-channel microelectrodes. *Biochem Eng BME* **21**:482-485.
2. Anderson, J.M. 1979. Structural studies on human spectrin. *J Biol Chem* **254**:939-944.
3. Anderson, J.M. 1979. Proteolytic fragmentation of spectrin: Effect of removal of terminal phosphopeptides on spectrin binding to human erythrocyte membrane. In: *Normal and abnormal red blood cell membranes*. Eds. S.E. Lux, V.T Marchesi, C.F. Fox, Allan Liss Inc. New York, pp. 531-534.
4. Anderson, J.M. and J.M. Tyler. 1980. State of spectrin phosphorylation does not affect erythrocyte shape or spectrin binding to erythrocyte membranes. *J Biol Chem* **255**:1259-1265.
5. Tyler, J.M., Anderson, J.M. and D. Branton. 1980. Structural studies on several actin-binding macromolecules. *J Cell Biol* **85**:489-495.
6. Anderson, J.M., Stevenson, B.R., Jesaitis, L.A., Goodenough, D.A. and M.S. Mooseker. 1988. Characterization of ZO-1, a protein component of the tight junction from mouse liver and Madin-Darby canine kidney cells. *J Cell Biol* **106**:1141-1149.
7. Stevenson, B.R., Anderson, J.M., Goodenough, D.A. and M.S. Mooseker. 1988. Tight junction structure and ZO-1 content are identical in two strains of Madin-Darby canine kidney cells which differ in transepithelial resistance. *J Cell Biol* **107**:2401-2408.
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14. Schnabel, E., Anderson, J.M. and M.G. Farquhar. 1990. The tight junction protein ZO-1 is concentrated along slit diaphragms of the glomerular epithelium. *J Cell Biol* **111**:1255-1264.
15. Watson, P.M., Anderson, J.M., Van Itallie, C.M., and S.R. Doctrow. 1991. The tight junction-specific protein ZO-1 is a component of the human and rat blood-brain barrier. *Neuroscience Letter* **129**:6-10.

James M. Anderson, Ph.D., M.D.

16. Byers, S.W., Citi, S., Anderson, J.M., and B. Hoxter. 1992. Polarized functions and permeability properties of rat epididymal epithelial cells in vitro. *J Reproduction & Fertility* **95(2)**:385-396.
17. Willott, E., Balda, S.M., Heintzelman, M., Jameson, B. and J.M. Anderson. 1992. Localization and differential expression of two isoforms of the tight junction protein ZO-1. *Am J Physiol* **262**(Cell Physiol 31):C1119-C1124.
18. Kurihara, H., Anderson, J.M., Keraschki, D., and M.G. Farquhar. 1992. The altered glomerular filtration slits seen in purimycin aminonucleoside nephrosis and protamine sulfate-treated rats contain the tight junction protein ZO-1. *Am J Path* **141(4)**:805-816.
19. Kurihara, H., Anderson, J.M., and M. Farquhar. 1992. Diversity among tight junctions in the rat kidney: The glomerular slit diaphragms and endothelial junctions express one not both isoforms of the tight junction protein ZO-1. *Proc Natl Acad Sci, USA* **89**:7075-7079.
20. Madara, J.L., Carlson, S. and J.M. Anderson. 1993. The tight junction protein ZO-1 maintains its spatial distribution but "dissociates" from junctional fibrils during tight junction regulation. *Am J Physiol* **264**(Cell Physiol):C1096-C1101.
21. Balda, M.S. and J.M. Anderson. 1993. Two classes of tight junctions revealed by ZO-1 isoforms. *Am J Physiol* **264** (Cell Physiol):C918-C924.
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James M. Anderson, Ph.D., M.D.

32. Fallon, M.B., Nathanson, M.H., Mennone, A., Sáez and J.M. Anderson. 1995. Altered expression and function of hepatocyte gap junctions following common bile duct ligation. *Am J Physiol (Cell)* **268**:C1186-94.
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# Computation Book

Number of Book \_\_\_\_\_

Name C. Van Itallie

Subject LMP 100 X5-4

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Plasmid prep of 1, 5, 7 for sequencing, Northerns etc

OD 5<sub>ul</sub> / 1000<sub>ul</sub>

260 280

#1	0.320	0.173	1.8	2.56 $\mu$ g/ul
5	0.496	0.276	1.8	7.0 $\mu$ g/ul
7	0.328	0.189	1.7	2.6 $\mu$ g/ul

use 10x digests - dig (for seq) more for insert prep

1<sup>st</sup>

- #1, 5, 7 1<sub>ul</sub> plasmid mix - 1<sub>ul</sub> 10X RI buffer

9<sub>ul</sub> water

0.4<sub>ul</sub> BSA

3<sub>ul</sub> RI

28.6<sub>ul</sub> H<sub>2</sub>O

also - 20<sub>ul</sub> for insert prep

#1 8<sub>ul</sub> plasmid

10<sub>ul</sub> 10X enzymes

1<sub>ul</sub> BSA

3<sub>ul</sub> EcoR I

7.8<sub>ul</sub> H<sub>2</sub>O

#5 8<sub>ul</sub> plasmid

10<sub>ul</sub> 10X buffer

1<sub>ul</sub> BSA

3<sub>ul</sub> Bam HI

3<sub>ul</sub> Hinc II

7.5<sub>ul</sub> H<sub>2</sub>O

#5 5<sub>ul</sub> plasmid

10<sub>ul</sub> 10X buffer

1<sub>ul</sub> BSA

3<sub>ul</sub> Hinc II

8<sub>ul</sub> H<sub>2</sub>O

Take over for sequencing

#5 - T3, T7

#1 - 18684 (3.2<sub>ul</sub>) 18686

#7 - 18689, 18685 3.

18684 - antisense strand #2

18685 - sense 341-365 Ja 7 OCT 7

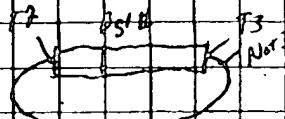
18686 - 344-361 Ja 1 OCT 7

cut O.N.

P.C. extract

Place full length vector clone for expression

Plan: cut clone I, 7 w Bgl II, Not I



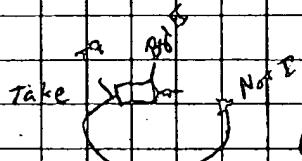
K<sup>+</sup> Not I because #1 → past stop but finds 5' end

Clone 1 2.6 μg/μl

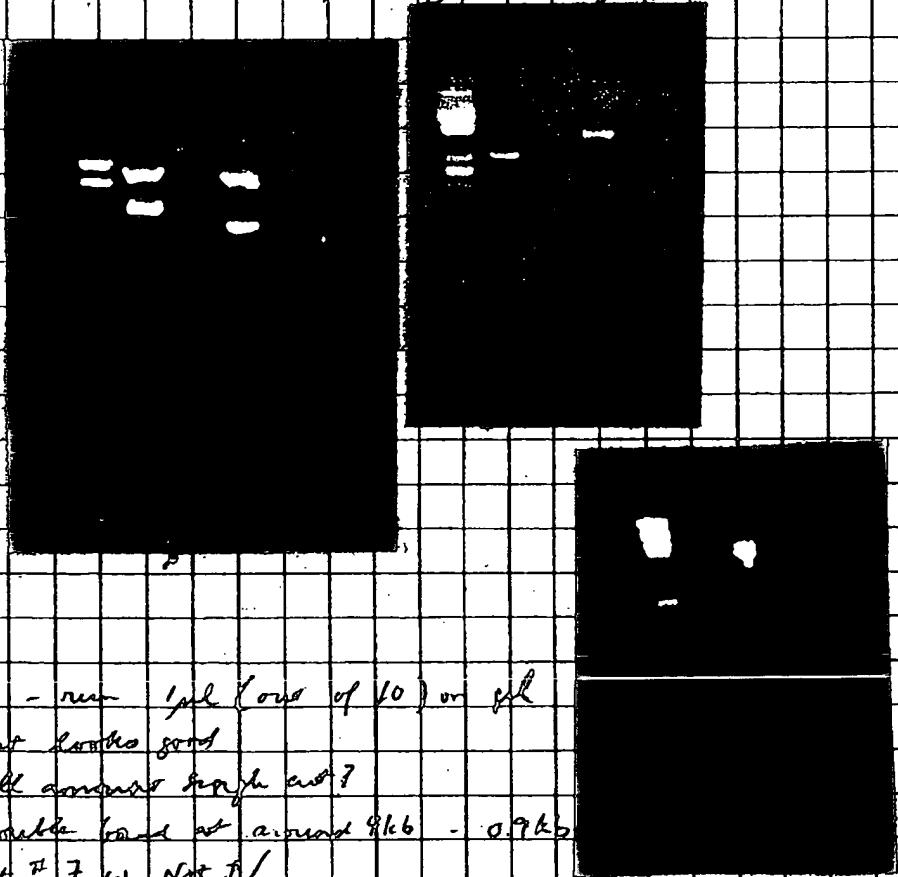
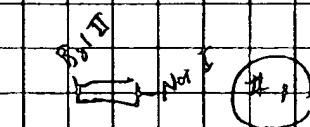
7 "

cut 10 μg of clone #7

20 μg of clone #1



cut ✓ 4 μl planned ✓ 8 μl planed  
10 μl 10x 3 10 μl 10x 3  
✓ 1 μl BSA ✓ 1 μl BSA  
2 μl Bgl II 2 μl Bgl II  
2 μl Not I 2 μl Not I  
✓ 81 μl H<sub>2</sub>O ✓ 77 μl H<sub>2</sub>O



Gene clear #1, 7 - run 1 μl (out of 10) on gel

result - #1 doesn't look so good

#7 - small amounts though out?

double band at around 0.9 kb - 0.9 kb

recut #7 w Not I /

Not I/Bgl II

6 μl H<sub>2</sub>O

run #2

(1)

4 μl plasmid

(2)

(3)

old prep

(4)

Resolution of human ocularis sequence published and  
have been working in wrong clone - clone #1 is correct  
so part of anti ACRI recom (Kpn) Xba

cut clone #1 w Kpn  
- 10 μl #1

10 μl 10x buffer #1

1μl 8A

3rd enzyme

67 μl H<sub>2</sub>O

cut pC8G w Xba I

clone #2.1 w Spe I

start at 12:58 PM - 2:30

run a gel for gene clean

Kpn I/Spe I insert is 2.45

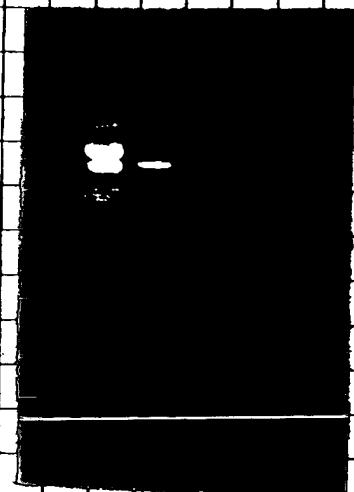
plasmid is 2.9

pC8G - 6.2

gene clean



] run for 60' at 120V 2:30 - 3:30



Gel image

①

Gel image

Gel vector

2 μl 10x 1.4

1 μl 8A

17.20 H<sub>2</sub>O

Gel image

2 μl Pst vector

2 μl 10x 1.4

1 μl 8A

13 μl H<sub>2</sub>O

③

Gel Pst vector

2 μl 10x 1.4

1 μl 8A

16 μl H<sub>2</sub>O

④

Gel vector

2 μl 10x 1.4

1 μl 8A

14 μl H<sub>2</sub>O

Paste gene r

2nd gene

2nd 10x

2nd Pst

14 μl H<sub>2</sub>O

3rd 32P

2nd 10x

take 2

#2, 3

transform

on

Gel Li

10 μl cell culture

30' 100